The Clinical Relevance of Chromogranin A as a Biomarker for Gastroenteropancreatic Neuroendocrine Tumors

Ben Lawrence, MBChB, MSc[a], Bjorn I. Gustafsson, MD, PhD[b,c],
Mark Kidd, PhD[a], Marianne Pavel, MD[d], Bernhard Svejda, MD[a],
Irvin M. Modlin, MD, PhD, DSc, FRCS(Eng & Ed)[a,*]

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THE BIOCHEMICAL DIAGNOSIS OF NEUROENDOCRINE TUMORS

An accurate tumor marker is a critical tool in tumor management because it establishes an uncertain diagnosis, offers a basis for individual prognostication, signals response to therapy, and identifies relapse. In classical terms, a high-quality tumor marker should represent a biologic attribute unique to the tumor cell or its local environment. Although this has proved manageable in a homogenous tumor population, the goal has been difficult to attain in gastroenteropancreatic neuroendocrine tumors (GEP-NETs) because they comprise an extremely heterogeneous group of cancers. Thus, the conundrum of identifying a global marker for NETs has remained a considerable technical challenge.

The identification of chromogranin A (CgA) in secretory vesicles in the adrenal medulla,[1] the development of a specific antibody,[2] and localization to extra-adrenal neuroendocrine cells[3,4] provided a partial solution. The clinical utility of this tool is blunted, however, by the ubiquity of CgA in normal tissue, the variable methodology

[a] Gastrointestinal Pathobiology Research Group, Department of Surgery, Yale University School of Medicine, 310 Cedar Street, PO Box 208602, New Haven, CT 06520-8062, USA
[b] Department of Gastroenterology, St Olavs Hospital, 7006 Trondheim, Norway
[c] Department of Cancer Research and Molecular Medicine, Norwegian University of Science and Technology, Prinsesse Kristinas Gate 1, 7006 Trondheim, Norway
[d] Med. Klinik m.S. Hepatologie und Gastroenterologie, Charité - Campus Virchow Klinikum, Universitätsmedizin Berlin, Augustenburger Platz 1, 13353 Berlin, Germany
* Corresponding author. Department of Gastroenterological Surgery, Yale University School of Medicine, 333 Cedar Street, PO Box 208062, New Haven, CT 06520-8062.
E-mail address: imodlin@optonline.net

of its measurement, and the diverse disease processes and physiologic events that perturb the granin family of peptides. To assess the utility of CgA measurement for clinical application, a basic understanding of essential CgA biology is necessary. This article provides an overview of the strengths and limitations of CgA as a tumor marker. It encompasses the physiologic role of CgA and evaluates the causes of CgA elevation in non-NET disease states and assesses the test platform variations and the impact on clinical care of NETs.

THE BIOLOGY OF CHROMOGRANIN A IN NORMAL CELLS
The Neuroendocrine Cell

Neuroendocrine cells aggregate in classical endocrine glands (eg, adrenal, pituitary, and parathyroid) but in the diffuse neuroendocrine system (DNES)—the diaphanous, ill-defined, and poorly understood neuroendocrine syncytium integrated throughout the bronchopulmonary and gastrointestinal (GI) systems.[5] Although the overarching role of the DNES as a wide-ranging regulator of secretion, absorption, and motility is broadly understood, the precise mechanistic basis of its function and its cell lineage remains, for the most part, opaque.

The cellular origins of GEP-NETs are diverse and reflect the numerous neuroendocrine cell types of the DNES in the GI tract.[6] Certain neuroendocrine cells are localized to a single organ (eg, gastric enterochromaffin-like [ECL] cells) whereas others (eg, the enterochromaffin [EC] cells), are ubiquitous throughout the GI tract (**Table 1**). Neuroendocrine cells share several common features, including production of secretory granules, maturation, and exocytosis as well as the synthesis of specific proteins and the presence of electron-dense or translucent secretory granules that are prototypical of the neuroendocrine cell type.[6] Of particular interest is the synthesis and biologic role of the granin family of proteins and peptides, especially that of CgA.[7]

The Granin Family

Granins are found as major, or principal, components of the soluble core of dense-core secretory granules in neuroendocrine cells and are secreted in a physiologically regulated manner.[8–11] There are 8 members in granin family, including CgA, CgB, CgC (secretogranin [Sg] II), SgIII, SgIV, SgV (7B2), Sg VI (NESP55), and VGF nerve growth factor–inducible (VGF) (**Fig. 1**). Granins have been proposed as playing important roles in secretory granule formation, processing, and development. The precise function, however, of individual granins is dependent on the presence of other granins and hormones produced by a specific neuroendocrine cell, the presence of proteolytic processing enzymes, and their inhibitors and activators as well as the density and localization of calcium pumps and exchangers. Of critical relevance to their clinical utility is the observation that irrespective of the cellular type, processing milieu, or expression of other granins, all are cosecreted with a variety of peptide and amine hormones depending on the neuroendocrine cell type.

Neuroendocrine Cell Types and CgA Secretion

Each neuroendocrine cell type in the DNES produces different amines, peptides, and proteins with a variety of biologic functions. At the same time, neuroendocrine cells cosecrete CgA during the secretory granule exocytotic process. Based on this biologic event, CgA has come to represent a common denominator peptide with the putative ability to be a marker of neuroendocrine cell activity (see **Table 1**). CgA was the initial member of the granin family identified, and its name represents the

Table 1
GEP-NET cell types and CgA secretion

Organ	Cell Type	Active Peptide	Related Tumor	Positive CgA Immunohistochemistry
Stomach—fundus	ECL cell	Histamine	ECLoma (types I, II, III)	Yes[63]
Antrum	X cell	Amylin	—	—
Antrum (and duodenum)	G cell	Gastrin	Gastrinoma	Yes[63]
Duodenum	I cell	CCK	CCKoma	Yes[145]
	S cell	Secretin	—	Yes[145]
	M cell	Motilin	—	No[145,146]
Duodenum/jejenum	K cell	GIP	GIPoma	Yes[145]
Small intestine	L cell	GLP1, PYY, NPY	—	Yes[145]
	N cell	Neurotensin	—	Yes[145]
Pancreas	β cell	Insulin	Insulinoma	Yes[63]
	α cell	Glucagon	Glucagonoma	Yes[63]
	F cell	Pancreatic PP	PPoma	Yes[63]
Entire GI tract	EC cell	Serotonin, substance P, guanylin, melatonin	Carcinoid, SI-NET	Yes[63]
	D cell	Somatostatin	Somatostatinoma	Yes[104,145]
	VIP cell	Vasoactive intestinal peptide	VIPoma	Yes[147]

Abbreviations: CCK, cholecystokinin; SI-NET, small intestinal neuroendocrine tumor.
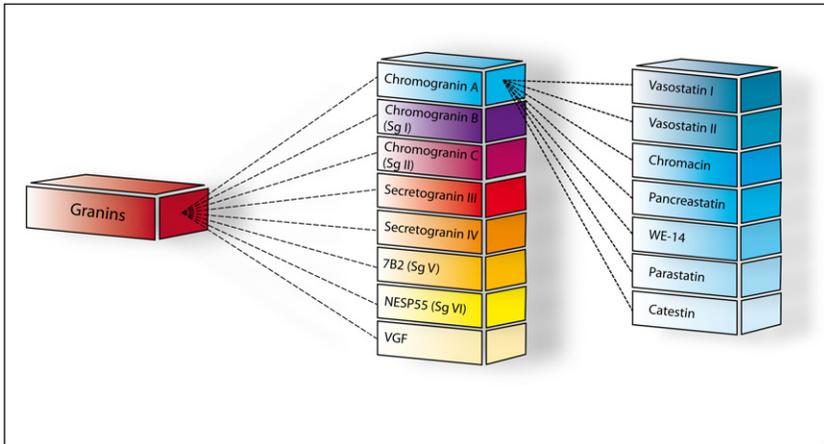
Fig. 1. The granin family and fragments of CgA. Neuroendocrine cells manufacture the 8 granin proteins in large dense-core secretory vesicles, including chromogranin (Cg) A, CgB, SgII (CgC), SgIII, SgIV, 7B2 (SgV), neuroendocrine secretory protein 55 (NESP55 or SgVI), and VGF nerve growth factor–inducible (VGF). CgA may be present in the blood as a major constituent, or as a series of smaller biologically active peptides created by postsecretory processing (right-hand box stack). These peptides include pancreastatin, catestatin, vasostatin I and II, and others.

original detection in the catecholamine-containing chromaffin granules of the adrenal medulla.[1,12] It is encoded by the *CHGA/CgA* gene located on chromosome 14.

CgA mRNA and protein are pan-neuronally expressed,[13] which reflects the extent of dense-core granule formation in diverse cell types throughout the DNES. CgA expression generally correlates with dense-core granule number within a neuroendocrine cell. In PC12 and 6T3 neuroendocrine cells (rat pheochromocytoma and pituitary corticotroph cell lines, respectively), CgA is considered to function as on/off switch for the formation of large dense-core granules.[11] Sequence comparisons of CgA from various mammalian (eg, man, monkey, equine, pig, cattle, rat, and mouse), avian (ostrich *Struthio camelus*), and teleostean species have revealed considerable interspecies homology,[14–17] underlining the fundamental importance of CgA in cellular function.

Cleavage of CgA to Other Peptides

Granins serve as precursor proteins that can be proteolytically processed by prohormone or proprotein convertases at multiple cleavage sites to produce a large number of small bioactive peptides, with a wide range of proposed biologic activity.[8] CgA is a 439 amino acid protein[18] produced as a component of a complex processing mechanism; it may be present in the blood as a major constituent or as a series of smaller biologically active peptides, such as vasostatin I and II, chromacin, pancreastatin, WE14, parastatin, and catestatin (see **Fig. 1**). Evidence for biologic activity in granin-derived peptides has accumulated since pancreastatin was first demonstrated to inhibit insulin secretion in 1986.[19]

Physiologic Role of CgA and Peptide Fragments

Although a definitive function for the complete CgA protein remains to be determined, a range of biologic functions is mediated by CgA-derived peptides (**Figs. 2** and **3**). CgA can exist in a variety of molecular forms and has been proposed as subsuming
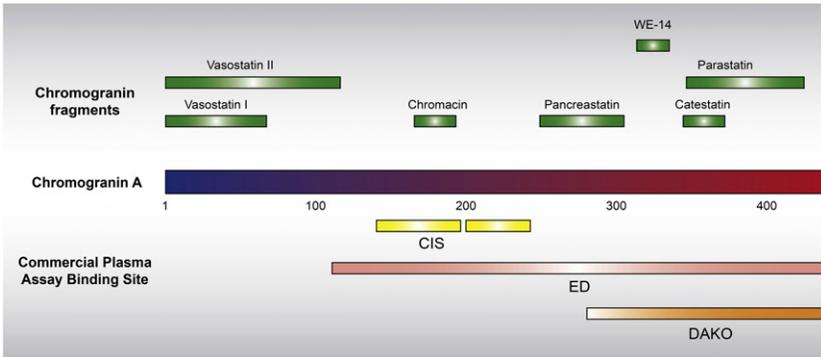
Fig. 2. The CgA protein, its peptide fragments, and the binding sites of the commercial p-CgA assays (CIS, ED, and DAKO). CgA is an approximately 460 amino acid protein that undergoes postsecretion processing into fragments, including pancreastatin (corresponding to human CgA residues 250–301), catestatin (residues 352–372), vasostatin I (residues 1–76), and vasostatin II (residues 1–113). The different sensitivities of 3 commercial CgA assays reflect the different binding epitopes of the antibody used in each test and the differential ability to bind to peptide fragments. The binding region of the DAKO antibody is not stated in product information, but because is standardized against a 23-kDa CgA fragment at the C- terminal, it will include at least some portion of the fragment indicated.

a diverse array of biofunctions ranging from neuroendocrine secretory regulation to cardiac function, vasomotor activity, and antimicrobial and antifungicidal activity to influences on intestinal smooth muscle contraction and modulatory roles in cell adhesion and homeostasis.[19–34] Possible additional roles include regulation of glomerular
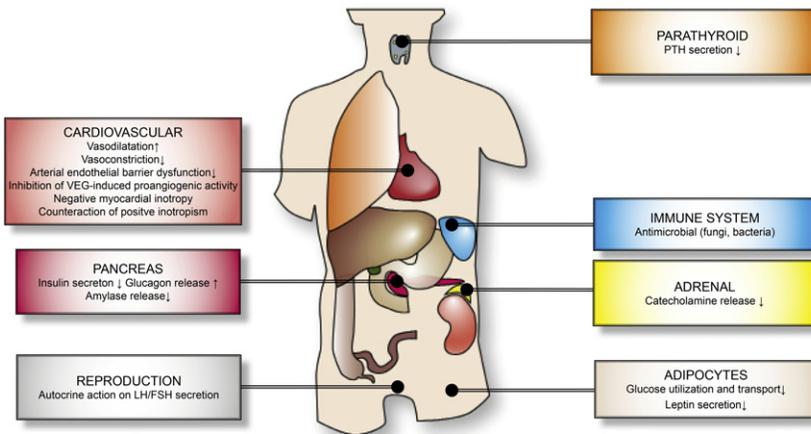

Fig. 3. Physiologic role of CgA. Although the definitive function for CgA itself remains unclear, CgA-derived peptides mediate a diverse array of biologic functions. These include regulation of parathyroid hormone secretion, carbohydrate metabolism, lipid metabolism, catecholamine secretion, several cardiovascular processes, immune properties, and reproduction. Variations in P-CgA may, therefore, reflect numerous functional alterations in a wide variety of biologic systems. In addition, CgA levels fluctuate on average by approximately 30% on repeated testing of normal individual and increase after food intake or physiologic stress.

filtration,[35] inflammation,[36–40] and microglial activation in the central nervous system.[41]

The physiologic role of many CgA peptide fragments is more certain. Vasostatins inhibit arterial vasoconstriction, including small and medium resistance vessels[22,23,25] as well as coronary and cerebral arteries,[30,42,43] and protect the heart against positive inotropism caused by β-adrenergic stimulation. Vasostatins also have antimicrobial properties by encouraging macrophage migration in inflammatory response,[27,29] inhibit PTH secretion,[25] and might regulate gonadotrophins.[44] Vasostatin I further inhibits tumor necrosis factor α–induced disruption of endothelial cells[45] and vascular endothelial growth factor.[46] Pancreastatin inhibits glucose-induced insulin release,[47] inhibits insulin-evoked glucose transport,[48] inhibits leptin secretion,[49] inhibits acid secretion from parietal cells,[50] and suppresses cholecystokinin-induced amylase secretion.[51] WE14 secretion might regulate gonadotrophins.[44] Parastatin inhibits PTH secretion,[24] inhibits pancreatic β-cells insulin release, and stimulates histamine release from ECL cells. Catestatin inhibits catecholamine release from adrenal chromaffin cells,[52] is coreleased with atrial natriuretic peptide,[53,54] has been proposed as protecting the heart against sympathetic overactivation,[54] and has antimicrobial activity against bacteria, fungi, and yeast.[55,56]

CGA MEASUREMENT

Based on post-translational processing (cleavage), CgA circulates as a highly heterogeneous antigen composition—comprising complete protein or constituent fragments. The efficacies of antibodies used in a particular CgA immunoassay therefore are of considerable relevance (see **Fig. 2**). CgA processing varies between different neuroendocrine organs, such that there is more extensive cleavage of CgA in pancreatic islets than in the adrenals, and different fragment profiles exist for each of the pancreatic α, β, D, and PP cells.[57]

CgA Testing Platforms for Blood

There are several commercially available and laboratory-developed assays for the measurement of circulating CgA concentrations. Three examples of commercial CgA assay kits are CgA-RIA CT (CIS bio international, Gif-sur-Yvette Cedex, France), DAKO Chromogranin A ELISA Kit (DAKO A/S, Glostrup, Denmark), and CgA EuroDiagnostica (Malmö, Sweden). These kits differ in methodologic techniques, such as radioimmunoassay and enzyme-linked immunosorbent assay (ELISA), have different standardization, and use different antibodies and binding epitopes (see **Fig. 2**). The calculated CgA level subsequently varies broadly between test platforms, with varying sensitivity and specificity.[58] Coefficients of variation also differ between testing kits.[59] At present, no universally accepted CgA assay exists and caution should be exhibited in the comparison of CgA concentration undertaken in different sites using different assay techniques.

CgA can be measured in plasma CgA (P-CgA) or serum CgA (S-CgA) without significantly changing the CgA level. A comparison of P-CgA to S-CgA concentration identified a strong positive linear relationship between these two measures (r = 0.9858, $P<.0001$), indicating CgA measurement can be undertaken in either sample type.[60] Although CgA measurement in saliva has been analyzed as a measure of stress response, this compartment has not, however, been investigated in NET patients.[61,62]

CgA Testing Platforms for Tissue

Immunohistochemical staining for CgA and synaptophysin is regarded as standard for a histopathologic diagnosis of NETs. CgA staining can be achieved in many neuroendocrine cell types, including pancreatic α, β, and polypeptide (PP) cells; gut EC, ECL, and G cells; thyroid C cells; parathyroid cells; adrenal medullary cells; and pituitary thyroid-stimulating hormone, follicle-stimulating hormone, and luteinizing hormone cells as well as some axons of visceral nerves.[63] An increased sensitivity in CgA-positive tumor detection by immunohistochemistry can be achieved by tyramide signal amplification, which is of value in diagnosing dedifferentiated neuroendocrine carcinomas.[64,65] Immunohistochemical staining for the NESP55 fragment (as well as CDX-2, PDX-1, and TTF-1) can help distinguish GI-NETs from pancreatic endocrine and pulmonary NETs.[66]

Several different CgA antibodies are commercially available for histopathologic use. Each antibody has variable sensitivity for different neuroendocrine cell types, for example, in the different pancreatic neuroendocrine cells. The monoclonal antibodies clone, LK2H10 (eg, Roche Molecular Biochemicals, Mannheim, Germany), is widely used for routine histopathology, has binding epitopes located in the CgA residues 250–284 (the N-terminal part of pancreastatin), and stains normal islet α cells well and β cells weakly but is nonreactive for D and PP cells.[57] The DAKO polyclonal antibody (A-0430) binds to the C-terminal part of the CgA molecule, has a broader range of epitopes, and may provide positive immunoreactivity in tumors where the monoclonal antibody is negative. The CgA 176–195 antibody[67] against the CgA midportion, however, displays strong immunoreactivity in all islet cell types except D cells and was the only CgA antibody expressed in all NETs, making it a superior pancreas endocrine cell and tumor marker compared with the other CgA antibodies.[68] Similar variability should be expected when staining neuroendocrine cells outside the pancreas.

A systematic immunocytochemical investigation of CgA fragments demonstrated expression of a more extensive variety of CgA fragments in NETs than normal neuroendocrine cells.[68] The CgA fragment pattern may thus be of value in evaluating the biologic behavior of NETs and region-specific antibodies of potential use when differentiating between benign and malignant NET types.[68] Staining variation is also exhibited by NETs of different histologic grade. The staining intensity of CgA antibodies is high in well-differentiated NETs and is comparable to staining in a normal neuroendocrine cell. Conversely, poorly differentiated neuroendocrine carcinomas (PDECs) are often nonimmunoreactive for CgA because of the rarity of large, dense-core granules.[69] The loss of CgA expression in PDECs indicates their incomplete or partial endocrine differentiation, in keeping with the on/off switch function of the *CHGA/CgA* gene for endocrine differentiation in mammalian cells.[11] Thus, P-CgA, which is related to the hormone's expression in the granules, may be within the reference interval or only slightly elevated in PDECs.[69]

PATHOLOGIC ELEVATION OF CGA
Nonmalignant but Pathologic Causes of Elevated CgA

As a consequence of the ubiquitous cosecretion of CgA with other regulatory peptides (see **Table 1**), there are multiple causes of CgA elevation that are unrelated to NETs. CgA elevation is associated with diverse GI, cardiovascular, pulmonary, rheumatologic, and endocrine diseases (**Fig. 4**).[36,38,70–72] CgA elevation in GI disease occurs in chronic atrophic gastritis,[73] liver cirrhosis, chronic hepatitis,[71] pancreatitis,[74] inflammatory bowel disease,[75] *Helicobacter pylori* infection,[76] and even irritable bowel syndrome (**Table 2**).[77,78] The elevated levels of CgA in inflammatory bowel disease
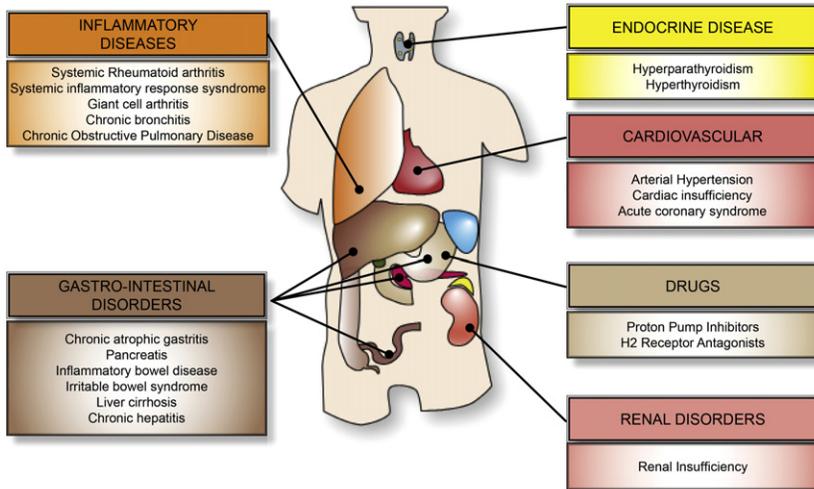
Fig. 4. Non-neoplastic causes of CgA elevation. CgA is elevated in endocrine diseases, chronic and acute inflammation, and cardiac insufficiency. Acid-suppressive medications result in hypergastrinemia (G-cell and ECL-cell hyperplasia) and a concomitant increase in cosecreted CgA. Renal failure increases detectable p-CgA by reducing glomerular filtration of CgA-related peptides. P-CgA alone cannot discriminate between GEP-NETs, pancreatitis, inflammatory bowel disease, irritable bowel syndrome, or hepatitis.

are of particular interest because the incidence of GI-NETs is significantly elevated in inflammatory bowel disease, possibly reflecting increased neuroendocrine cell proliferation in an inflammatory milieu.[79]

Elevated levels of circulating CgA as a consequence of non-GI disease occur in patients with hypertension,[80–82] heart failure,[37,83] renal failure,[84,85] systemic inflammatory response syndrome,[86] hyperthyroidism,[87] pulmonary obstructive disease,[62,88]

Table 2
Sensitivity of elevated P-CgA levels in detection of non-neuroendocrine GEP disease

Disease/Disorder		Detection Sensitivity (%)
Non-neoplastic	Chronic atrophic gastritis[73]	78–100
	Pancreatitis[74]	23
	Inflammatory bowel disease[71,75]	28–55[a]
	Irritable bowel syndrome[77]	20–31[b]
	Liver cirrhosis[71]	19–48
	Chronic hepatitis[71]	20
Neoplastic	Colon cancer[148]	1–20
	HCC[40]	70–83
	Pancreatic adenocarcinoma[74]	43–83
Drugs	PPIs[58,149]	100[c]
	H$_2$ blockers[92,150,151]	0–8

[a] In active disease using a cutoff value of >20 μ/L.
[b] Normal range 0–20 μ/L.
[c] Medium (6 weeks–1 year) and long-term treatment (1–8 years).

and exercise-induced physical stress.[89] Furthermore, giant cell arteritis,[38] rheumatoid arthritis, and systemic lupus erythematosus have been associated with increased circulating concentrations of CgA.[88]

Iatrogenic Causes of CgA Elevation

The widespread use of the proton pump inhibitor (PPI) class of drugs and other acid-suppressive medications is a substantial cause of CgA elevation.[76,90,91] In the setting of PPI medication, the lack of gastric acid engenders hypergastrinemia, G-cell hyperplasia, and ECL cell hyperplasia, with both neuroendocrine cell types cosecreting CgA with their respective products (gastrin and histamine). Omeprazole therapy may engender CgA elevations that are in excess of 690 μg/L (mean 45 ± 18 μg/L [normal range: 16–97 μg/L]) and can occur as early as 6 days after first intake of PPI.[91] Higher CgA levels are noted after long-term treatment (1–8 years) compared with midterm treatment (<1 year).[92] CgA concentration is higher with PPI usage compared with histamine type-2 receptor antagonist (H2RA).[92] Withdrawal of PPI leads to normalization of CgA within 1 to 2 weeks,[91] so elevated CgA concentration consequent on the use of acid-suppressive pharmacotherapy can be confirmed by withdrawal of the PPI for a period of 2 weeks followed by review of the CgA levels.

Malignant but Non-neuroendocrine Causes of CgA Elevation

Although increased circulating CgA concentrations are moderately sensitive markers of GI-NETs, they are not specific for a neuroendocrine malignancy.[93] CgA elevation in non-NET tumors usually reflects an underlying pattern of neuroendocrine differentiation (eg, pancreatic, colorectal, gastric, or prostate adenocarcinoma), although in HCC or breast cancer, the cause is unclear.[94–96]

Underlying neuroendocrine differentiation has been postulated to occur in several cancers, although CgA is only expressed in cell nests within these cancers and is not considered an adequate humoral tumor marker when compared with standard tumor markers for these cancers. Neuroendocrine differentiation is not an uncommon event in primary colorectal cancer (34%) and these patients exhibit a worse prognosis after routine surgical therapy.[97] This suggests that neuroendocrine differentiation correlates with a more aggressive disease course and reflects the observation that neuroendocrine differentiation is often identifiable in small cell undifferentiated colorectal cancer.[98] Prostatic and pancreatic adenocarcinomas also exhibit a neuroendocrine component, with the incidence of these cells in prostatic adenocarcinomas ranging from 10% to 100%. There is a positive correlation between the proportion of prostate cancer cells that stain for CgA and the serum levels of CgA,[95] and elevated CgA has been reported as indicative of a poor prognosis in localized[99] and metastatic[100] prostate carcinomas. In patients with pancreatic adenocarcinoma, mean CgA levels were significantly higher (192.9 ± 66.5 ng/mL) as compared with a group of healthy subjects (36.0 ± 22.0 ng/mL) and those with chronic pancreatitis (96.0 ± 31.0 ng/mL).[74] As with prostate cancer, individuals with high CgA levels and pancreatic cancer exhibited a poorer prognosis and survival.

Elevation of CgA has been identified in hepatocellular cancer (HCC) but its pathophysiologic basis is unknown. Elevated CgA above normal values occurred in 83% of patients with HCC, well in excess of the rates in liver cirrhosis, chronic hepatitis, and inflammatory bowel disease (48%, 20%, and 33%, respectively).[71] Diagnostic accuracy is, however, inferior to α-fetoprotein,[40] which remains the tumor marker of choice for HCC.

The relationship between CgA expression and breast cancer is also uncertain. P-CgA elevation can occur in breast adenocarcinoma, even in the absence of

immunostaining for neuroendocrine markers (including CgA) in the tumor.[101] On the contrary, P-CgA was elevated in only 2 of 8 pathologically confirmed neuroendocrine carcinomas of the breast,[101] suggesting a limited role for circulating CgA in this cancer.

THE CLINICAL UTILITY OF CGA IN NEUROENDOCRINE TUMORS
Rationale for CgA as a Tumor Marker

CgA is used as an important and reliable broad-spectrum marker for immunohisto-chemical identification of normal and neoplastic neuroendocrine cells (**Fig. 5**). The major advantage of CgA is co-secretion by multiple different neuroendocrine cell types. The major difficulty that limits the accuracy of CgA as a tumor marker is extensive postsecretion processing into several fragments; the fragment pattern varies between tumor primary sites and individuals, and the commercially available tests have differing abilities to detect the fragments.

A reasonable approach to developing a tumor marker for NETs might be to measure a specific peptide or amine produced by the tumor; however, many of these tumor products are obscure, difficult to measure, and unreliable due to diurnal fluctuation that exceeds the variation caused by the tumor itself. The original neuroendocrine cell type is also not immediately obvious on routine pathologic testing, and therefore the peptide of interest is not always initially apparent. By comparison, CgA is present in most NETs and is relatively stable, and commercially available assays are available.

CgA concentrations have, therefore, become regarded as moderately sensitive but nonspecific markers of individual NETs. Increased CgA concentrations can be detected in GEP-NETs, bronchopulmonary NETs (including small cell lung cancer), pheochromocytomas, neuroblastomas, medullary thyroid carcinoma, and Merkel
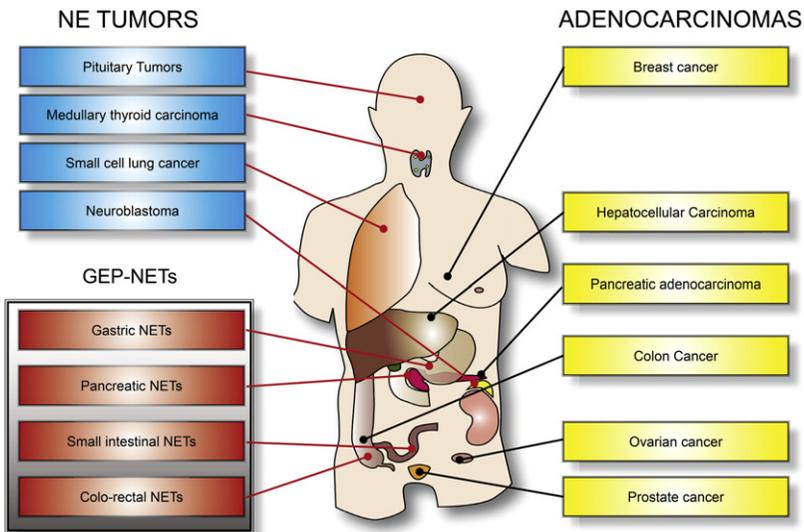
Fig. 5. Neoplastic causes of elevated CgA. CgA elevations occur in diverse NETs but are usually more pronounced in GEP-NETs (small intestinal, gastric, and pancreatic NETs). CgA elevations may occur in carcinomas with a complete or a partial neuroendocrine phenotype (left and right box stacks, respectively). In HCC, the cause of CgA elevation In HCC, the CgA elevation may reflect impaired metabolism of CgA fragments due to concurrent liver failure.

cell carcinoma of the skin.[58,69,93,102–118] CgA has limited use in diagnosis of pituitary adenomas where more specific biochemical markers (eg, prolactin, throid-stimulating hormone, luteinizing hormone, follicle-stimulating hormone, adrenocorticotrophic hormone, and insulinlike growth factor 1) are available.[107,119] Small cell lung carcinomas exhibit higher levels of CgA than any other type of lung cancer or healthy controls,[102] and the level is higher in extensive stage disease. CgB is a major granin of the human adrenal medulla,[120] plays a role in regulating secretion,[121] and may be a more sensitive marker for pheochromocytoma and related endocrine tumors than CgA or SgII.[122]

Sensitivity and Specificity of Elevated CgA

Diagnosis

Fluctuations in CgA concentration P-CgA levels vary in healthy control participants and patients with NETs; both eating and exercise lead to increases in CgA concentration. Levels are increased after eating in healthy controls and in multiple endocrine neoplasia type 1 (MEN-1) patients with or without a pancreatic NET (by 16%, 20%, and 31%, respectively).[70] The mean day-to-day variation of CgA in NET patients is 29.3% (range 0–113.5%), irrespective of a normal or elevated CgA or type of NET, and healthy subjects showed similar variability (21%, range 0%–47%). The maximum CgA values occur 30 to 60 minutes after eating and increase between 2- and 3-fold after meals.[90] Exercise-induced CgA elevation is evident as early as 2 minutes after high-intensity exercise and at 15 minutes postexercise and rises from a baseline value of 41 ± 10 μg/L to a peak of 56 ± 4 μg/L ($P<.005$).[123,124] To facilitate comparison and increase accuracy, CgA should, therefore, be measured in fasting patients and exercise should be avoided before the testing.

Comparison to alternative tumor markers for NETs Alternative NET tumor markers quantify the primary secretory product of the malignant neuroendocrine cell (see **Table 1**). The most widely used tumor marker for serotonin secreting NETs has previously been 24-hour urine measurement of the serotonin metabolite, urinary 5-hydroxyindoleacetic acid (u5-HIAA). Elevation of u5-HIAA correlates with the intensity of radiolabeled somatostatin uptake (tumor:background ratio) on somatostatin receptor scintigraphy (SRS), and the same relationship is observed between SRS and CgA.[111] CgA, however, demonstrates higher diagnostic accuracy than either u5-HIAA or other markers, including neuron-specific enolase (NSE) and carcinoembryonic antigen, in distinguishing NETs from controls (**Table 3**).[125] CgA correlates better than u5-HIAA in regard to physical functioning and quality of life[126] and is more convenient than u5-HIAA measurement. The latter requires a 24-hour urine collection and a complex dietary regime abstention from tryptophan/serotonin-rich foods (eg, bananas, avocados, plums, eggplant, tomatoes, plantain, pineapples, and walnuts) for 3 days before the collection period. Both markers may be considered complementary, however. Many other neuroendocrine cell–specific biochemical markers, including insulin, gastrin, glucagon, vasoactive intestinal peptide, serotonin, bradykinin, substance P, neurotensin, human chorionic gonadotropin, neuropeptide K, and neuropeptide L and pancreatic PP, have been identified in association with GI-NETs; for the most part, few have the specificity or predictive value of CgA (or even u5-HIAA) and their measurement is often complex and usually expensive.

Tumor markers of more generic cellular processes have also been examined and CgA identifies NETs from other forms of cancer more effectively than NSE and the alpha subunit of glycoprotein hormones.[107] In a group of patients with GEP-NETs, immunoassays identified elevated CgA in 99% of patients and elevated CgB levels

Table 3
Sensitivity and specificity of CgA in the detection of NETs

Study	NET Group	Comparison Group	Test	Sensitivity (%)	Specificity (%)
Bajetta et al[125]	n = 127 GEP NETs	N = 103 Blood donors	CgA[a]	68	86
			NSE	33	100
			Urine 5HIAA	35	100
			CEA	15	91
Campana et al[152]	n = 238 GEP-NETs	N = 42 CAG N = 48 disease-free patients	CgA vs disease free	85	96
			CgA vs CAG and disease free	75	84
Nobels et al[107]	n = 211 NETs	n = 180 non-NET cancers	CgA[b]	53	93
			NSE	46	65
			α-SU	26	85
Cimitan et al[69]	N = 63 Lung and GEP-NETs	No control	CgA[a]	55	94
			SRS[c]	77	94
Namwongprom et al[106]	N = 125 NET	No control	CgA[d]	62	84
			SRS[e]	83	98
			CgA and SRS	93	81
Nehar et al[112]	N = 124 GEP-NETs	N = 34 MEN-1 N = 127 controls	CgA[f]	63	98

Abbreviations: α-SU, alpha subunit of glycoprotein hormones; CAG, with chronic atrophic gastritis; CEA, carcinoembryonic antigen.
[a] ELISA (Dako A/S, Glostrup, Denmark).
[b] Polyclonal radioimmunoassay (UCB, Brussels, Belgium).
[c] 111In-pentetreotide SRS.
[d] Enzyme immunoassay.
[e] Indium-111-DTPA-Phel-octreotide including whole-body images as well as single-photon emission CT and CT.
[f] CgA-RIA CT kit (Cis bio international, Gif-sur-Yvette Cedex, France).

in 86% of patients.[127] Overall, CgA (despite its limitations) seems a better tumor marker than u5-HIAA, NSE, CEA, and alpha subunit of glycoprotein.

It has been proposed that improvement in diagnostic sensitivity can be achieved by combining CgA with a second diagnostic test. Thus, CgA and SRS detect NETs with approximately 60% and approximately 80% sensitivity, respectively,[69,106] which can be increased, by combining both tests, to 93% diagnostic sensitivity (see **Table 3**).[106] Combining CgA and pancreatic PP concentrations enhances the sensitivity in diagnosis of overall GEP-NETs, as well as in both functioning and nonfunctioning endocrine pancreatic tumors, to greater than 95%.[128]

Surveillance for pancreatic NETs is an important clinical issue in MEN-1. Identification of substantially elevated CgA levels strongly suggests the presence of an occult GEP-NET (especially concomitant gastrinoma in Zollinger-Ellison syndrome) because primary hyperparathyroidism or pituitary adenomas rarely are associated with marked CgA increases.[119,129,130] Thus, CgA not only is an important tool not only for the diagnosis of sporadic NETs but also is of value in MEN-1.

Prognosis and tumor burden

There is a substantial but indirect rationale for a prognostic role of CgA because it has some correlation to tumor stage, and advanced staging predicts diminished survival.[131] CgA levels are higher with extensive metastases than with localized disease or even limited hepatic involvement.[114,116,132] An investigation of 124 patients with sporadic GEP-NETs indicated that the degree of elevation of CgA levels reflected disease extent, with higher CgA levels occurring in metastatic compared with localized disease.[112] This relationship altered the sensitivity of CgA as a diagnostic tool with CgA apparently more sensitive in metastatic (73%) as opposed to localized disease (26%; $P<.01$).[112] Thus, CgA exhibits a relationship with the extent of hepatic tumor burden.[133]

An association between CgA and survival has been observed retrospectively in patients with metastatic GEP-NETs,[133] pancreatic NETs,[110] and nonfunctioning pancreatic NETs.[132] A CgA concentration 3 times above the upper normal limit at diagnosis is a significant predictor of shorter survival (hazard ratio 2.6) in patients with GEP-NETs.[110] In midgut carcinoid patients, an increase of CgA (>5000 μg/L) was an independent predictor of shorter survival,[116] and those with CgA less than 5000 μg/L had a significantly longer median survival (57 months) as compared with those with higher values (33 months). Elevated CgA levels were associated with a significantly poorer survival in 39 patients with midgut tumors with liver metastases treated with long-acting octreotide,[126] whereas no significant association was observed between 5-HIAA levels and survival time.

The relationship between CgA elevation and extent of disease is, however, not evident in all NET phenotypes. Gastrinomas are associated with high circulating CgA values even in the absence of liver metastasis, most likely representing the dual impact of tumor gastrin-associated CgA secretion and gastrin-driven ECL-cell histamine release. Also, some studies have noted an association between CgA and tumor location that is not always correlated with survival.[134] The highest maximum CgA values have been reported in ileal NETs (200 times the normal upper limit) and GEP-NETs associated with MEN-1 (150 times the normal upper limit). Types II and III gastric ECLomas (80–100 times normal) and pancreatic NETs and Zollinger-Ellison syndrome (in MEN-1) had intermediate values (60–80 times the upper limit of normal). In a variety of NETs (n = 211), CgA was found most frequently increased in gastrinomas (100%), followed by pheochromocytomas (90%), NETs (80%), nonfunctioning pancreatic NETs (70%), and medullary thyroid carcinomas (50%).[107] These findings

directly contradict the proposal that CgA concentration correlates positively with diminished survival.

An additional problem with CgA as a prognostic indicator occurs in the relationship with tumor grade. Because higher tumor grade is related to poorer survival,[131] it might be predicted that CgA should be higher in high-grade tumors if it was accurately prognostic. CgA is more frequently elevated in well-differentiated tumors, however, as compared with poorly differentiated tumors. This may reflect the functional integrity of the neuroendocrine secretory system in individual tumors or the individual secretory profile of the small bowel EC cell per se. In a study of 63 NETs, the diagnostic accuracy of CgA was 76% for well-differentiated NETs, 68% for well-differentiated neuroendocrine carcinomas, and 50% for PDECs.[69] None of the poorly differentiated NETs in this study occurred in the GI system. Nonetheless, it is apparent that overall, CgA provides only limited prognostic information, because, although it generally correlates with tumor burden, it does not consistently correspond to tumor grade. Thus, CgA is a useful tumor marker in well-differentiated NEC but is of less reliability in PDEC.

Response to treatment

Theoretically, it might be considered that a correlation between CgA and tumor burden should culminate in a reduction in CgA after successful therapeutic intervention. Simplistic validation of the relationship between CgA concentration and tumor bulk is confirmed by a reduction in CgA after surgical resection.[112] This provides the rationale for monitoring more speculative pharmacotherapeutic intervention. Clinical response to medical therapy has been reported with stable or reduced CgA in GEP-NETs[93,125,135,136] and in gastric carcinoids.[137–141] Reduction in CgA is observed after successful peptide receptor radionuclide therapy[142] and liver transplantation.[143] Overall, it seems that CgA has some degree of utility in monitoring therapeutic response with the proviso that an elevation must be detectable before intervention.

Relapse

A critical role of a tumor marker is to identify disease recurrence after definitive or palliative therapeutic intervention, especially when further therapeutic options may need to be initiated. CgA is well correlated to the tumor burden and, therefore, may be considered a moderately useful tool in NET treatment surveillance.[137] In patients followed up for radically operated midgut carcinoids, CgA was reported to represent the first indication of recurrence, ahead of u5-HIAA and traditional radiologic examinations (transabdominal ultrasound, CT, or MRI).[144] Specifically, CgA became pathologically elevated in 85% of patients who ultimately relapsed (in 33 of 56 patients after a median of 32 months).[144] The authors of this study concluded that in asymptomatic patients who underwent resection of a midgut NET primary, follow-up should only comprise measurement of CgA twice a year and annual transabdominal ultrasonography.[144] Further support for the value of CgA in detecting recurrence was reported in a heterogeneous group of NETs, where an elevation of CgA was identified in 83% undergoing clinical progression and in 100% of patients with progressive liver metastases.[125] Similarly, concordance with tumor progression was 81% in a large group of GEP-NETs and concordance was higher for CgA than levels of serotonin (54%).[112] It seems that CgA is a useful tool in the detection of disease relapse in selected patient groups, although further characterization of subgroups is required to delineate NET specific follow-up protocols.
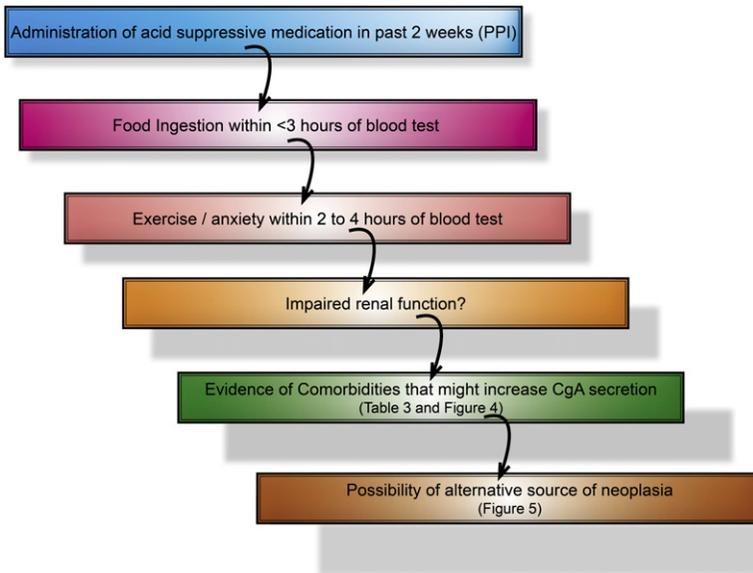
Fig. 6. A clinical checklist effective in ascertaining that detectable elevation in CgA identifies a neuroendocrine cancer. Although not exhaustive, this aide memoire summarizes the key issues clinically relevant to a clinician seeking to determine that an elevated CgA represents NET disease. The most common cause of CgA elevation is administration of PPIs, or the presence of either chronic atrophic gastritis or renal failure.

SUMMARY

CgA, although it exhibits limitations, is currently the most useful general tumor biomarker available for use in the diagnosis and management of GEP-NETs. The value of the CgA lies in its universal cosecretion by the majority of neuroendocrine cells that persists after malignant transformation. The added utility provided by the measurement of specific primary tumor–type biomarkers is rarely of prognostic value. The limitations of CgA measurement include a modest sensitivity of 50% to 60% when all stages and grades are considered and perturbation of the analysis by numerous physiologic and pathologic events unrelated to neuroendocrine cancer (**Fig. 6**). CgA also has mild to modest prognostic value in GEP-NETs, although some NET subtypes produce higher overall levels irrespective of prognosis. Circulating CgA is not useful as a prognostic tool in poorly differentiated tumors. Alteration of CgA levels has some utility in monitoring tumor relapse or progression. Clinicians aware of the physiologic role of CgA and its diverse implications in a variety of non-NET related pathologic conditions can adequately use this protein as a moderately effective tumor biomarker in the management of GEP-NETs.

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